関東森林研究 63-1(2012)

Temporal variation in airborne pollen density and genetic diversity of the pollen pool in *Fagus crenata*

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Abstract: The present study examined temporal variation in airborne pollen density and genetic diversity of pollen pool in *Fagus crenata*. Airborne pollen density of a *F. crenata* population in Gero City, Gifu Prefecture, Japan was observed at heights of 2, 12 and 22 m in an observation tower for a period of 11 days. Comparatively high airborne pollen density levels were observed for a period of 5 days during the study. In addition, observed pollen density was found to be highest at a height of 22 m. We put 18 of crossing bag for branches of a tree, and a few of those crossing bag was removed in only two days within flowering period. Nuts produced on those branches were collected, germinated, and then examined genetic diversity of the pollen pool during two periods (May 1-2 and May 3-4) using 7 pairs of SSR markers. The estimated number of pollen alleles varied between the two periods, with significant genetic differentiation in the pollen pool being detected using TwoGener analysis ($\Phi_{FT} = 0.136$). Genetic diversity of produced nuts may be promoted by temporal variations in genetic diversity of the pollen pool.

Keywords: Pollen pool, Temporal variation, SSR (Micoresatellite), Genetic diversity, *Fagus crenata*

I Introduction

Airborne pollen density at a fixed point tends to vary with time because factors related to pollen transfer, such as wind, change over time [4, 5]. Changes in pollen density can lead to changes in genetic diversity of the pollen pool; however, temporal variation in the genetic diversity of pollen pools has not yet been examined in wind-pollinated tree species. The pattern of pollen-mediated gene dispersal can be described as the sum of temporally variable patterns in pollen dispersal. Understanding the factors which lead to the determination of genetic diversity in the offspring of wind-pollinated tree species is important in relation to both breeding and genetic resource conservation. Revealing patterns of temporal variation in the pollen pool will contribute to the understanding of how diverse pollen-mediated gene dispersal patterns are produced.

The present study investigates temporal variation in airborne pollen density as well as genetic diversity of the pollen pool using *Fagus crenata* as a model species. *F. crenata* is a deciduous, broad-leaved, monoecious, wind-pollinated tree species and is widely distributed throughout Japan in cool temperate zones. In the pollen-mediated gene dispersal pattern of the *F. crenata* population examined in the present study, each mother tree has been shown to receive pollen from multiple fathers within the population [3].

The goals of this study were to reveal temporal variation of airborne pollen density within a population of *F. crenata* and to determine the genetic diversity of the pollen pool in a representative mother tree.

II Materials and Methods

1. Study site

The study site was located in the Kuraiyama experimental forest of Gifu field Science Center, Faculty of Applied Biological Sciences, Gifu University, in Gifu Prefecture, central Japan (36°00' N, 137°12' E; 1200 m above sea level). Approximately 9 ha of mixed deciduous forest are dominated by *F. crenata* and *Quercus mongolica* var. grosserrata and include about 500 *F. crenata* trees with diameter at breast height (DBH) > 10 cm. A 4 ha (160 × 250 m) study plot that included 143 *F. crenata* trees at the eastern edge of the study site was established. DBH of trees within study plot ranged from 10.0 to 89.5 cm, with an average of 43.9 cm. We confirmed that 108 of those 143 trees flowered in 2005.

2. Temporal variation in airborne pollen density

Three Durham pollen traps were set at heights of 2, 12 and 22 m

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above ground in an observation tower at the study site during 2005. The tower was located at the center of the 4 ha study plot. Glass slides with petrolatum were collected from each pollen trap every morning between 28 April and 12 May, 2005 and pollen density was measured using a light microscope. The number of pollen grains within three 0.2 × 0.2 mm areas on each slide were counted and used to estimate the number of pollen grains in a 1 cm^2 area. Unfortunately, the observation tower could not be accessed on 2 May due to rainfall the previous night. This meant that the glass slides collected on 3 May (i.e., the samples from which pollen density data for 2 May was calculated) included pollen from 1 May and 2 May. Observed pollen densities obtained from the 2 May sample were halved and used to represent values for both 1 May and 2 May. Hard rain prevented the collection of data from the 22 m trap on 6 May.

3. Observations of wind condition

An aerovane set 22 m above the ground in the observation tower was used to record wind direction and velocity every minute between 24 April and 13 May, 2005. This was recorded as an important environmental factor that influences pollen transfer.

4. Temporal variation in genetic diversity of the pollen pool

A tree standing beside the observation tower (DBH = 63.15 cm) was selected as a representative sample of *F. crenata* for use in the determination of pollen pool genetic diversity. A total of 18 branches on the sample specimen, each having more than 10 female flowers, were selected and placed in crossing bags prior to flowering. Three or four crossing bags were removed on 2 different days between 29 April and 8 May in order to allow pollination to occur after which point the bags were replaced. Seeds derived from exposed flowers were collected in September, germinated and had their DNA extracted from the cotyledon of seedlings using the DNeasy Plant Mini Kit (QIAGEN). Seeds were then genotyped using 7 pairs of microsatellite markers (*FS1-03*, *FS4-46*[5], *sfc0036*, *sfc0018*, *sfc0378*, *sfc1105*, and *sfc1143*;[1]) following methodology outlined in previous study[3].

TwoGener analysis[7] was used to analyze genetic differentiation of the pollen pool between two pollen receptive periods. TwoGener analysis is generally employed to analyze genetic differentiation of the pollen pool among mothers; however, in this study, the method was adopted to examine genetic differentiation of the pollen pool between two pollen receptive periods in a single mother tree. In addition, the genotypes of the mother and seedlings were compared, with only pollen alleles being detected and counted. When genotype of the mother tree and seedling was the same (i.e., a pollen allele could not be superficially identified), both alleles were counted as 0.5. This was accomplished using an Excel Macro program developed for use in this study (the tool used can be found at http://sfc1143.blog.fc2.com/blog-entry-1.html). Finally, the allele frequency and allelic richness of pollen alleles (*A*) and the number of private alleles (*PA*; alleles detected in either of the two sample periods) were calculated.

III Results

1. Temporal variation in airborne pollen density

F. crenata pollen grains were collected from 28 April to 8 May. Relatively high pollen density was observed from 3-7 May (Fig. 1) and airborne pollen density was at its highest 22 m above ground.

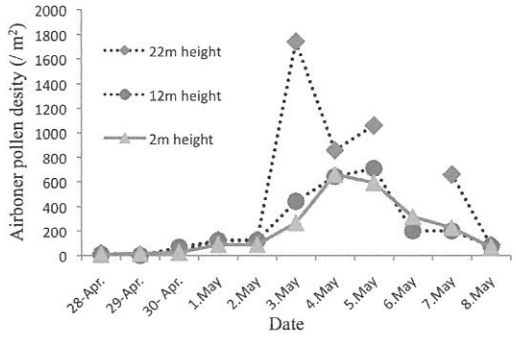

Fig. 1 Daily variation in airborne pollen density

2. Temporal variation in genetic diversity of the pollen pool

Although some samples were lost due to mold, relatively large numbers of seeds pollinated in the periods between 1 and 2 May (Period 1) and 3 and 4 May (Period 2) were germinated. Thirty-eight and 39 germinated seedlings were used for genotyping in period 1 and 2, respectively.

Significant genetic differentiation of the pollen pool (Φ_{FT}) was detected using TwoGener (0.136, $p < 0.01$). Allele frequencies within the pollen pool are presented in Fig. 2. Frequency distribution of each locus did not differ significantly (Fisher's exact test). Mean allelic richness of pollen alleles through 7

microsatellite loci was 8.86 and 8.00 for periods 1 and 2, respectively. Also, the numbers of private alleles for periods 1 and 2 were 19 and 13, respectively (Table 1). Genetic diversity of pollen alleles was higher in period 1.

Table 1. Results of genetic diversity of pollen pool.
N, the numbers of pollen allele; *A*, allelic richness of pollen allele; *PA*, the numbers of private allele of pollen pool.

	1-2 May			3-4 May		
Locus	*N*	*A*	*PA*	*N*	*A*	*PA*
FS3-01	10	10.00	5	5	5.00	0
FS4-04	6	6.00	2	6	6.00	2
sfc0018	8	8.00	2	8	8.00	2
sfc0036	10	10.00	2	10	10.00	2
sfc0378	10	10.00	4	7	7.00	1
sfc1105	7	7.00	2	9	9.00	4
sfc1143	11	11.00	2	11	11.00	2

3. Observations of wind condition

Northeasterly and south-southwesterly winds were dominant during the flowering period at the study site. Mean wind velocity differed between periods 1 (3.80 m/sec) and 2 (1.90 m/sec). Data regarding the wind conditions during both study periods is shown in Fig. 3.

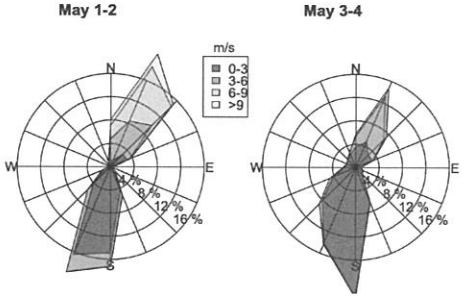

Fig.3 Observed wind direction and velocity within the study site during each of the two pollination period.

IV Discussion

1. Temporal variation in airborne pollen density.

The flowering period of *F. crenata* at the study site had a duration of 11 days (from 28 April to 8 May), but relatively high airborne pollen density was observed from 3 to7 May (Fig. 1). These results are consistent with the observed lengths of flowering in previous study[8]. Trapped pollen density was highest at 22 m, while densities at 2 and 12 m were similar. The crown of *F. crenata* is typically located at a height of approximately 20 m; therefore, the pollen trap 22 m above ground may have caught pollen grains more efficiently than those located at heights of 2 and 12 m. Previous studies have typically caught pollen grains at heights of approximately 1 m above ground. It should be noted that density and composition of the pollen pool at the height of tree crown might different.

2. Temporal variation in genetic diversity of the pollen pool

Pollen allele frequencies detected in period 1 and period 2 did not differ significantly (Fig. 2); however, significant genetic differentiation of the pollen pool was detected in TwoGener analysis (Φ_{FT}= 0.136).

Genetic diversity of the pollen pool was higher in period 1 than it was in period 2, with mean values of *A* and *PA* determined to be 8.86 and 19, and 8.00 and 13 for periods 1 and 2, respectively (Table 1). On the other hand, airborne pollen density observed during the study was lower in period 1 than it was in period 2 (Fig. 1). Results indicated that genetic diversity of the pollen pool was not necessarily proportional to airborne pollen density. Previous studies have demonstrated temporal variation in the pollen pool of the insect-pollinated tree species *Eucalyptus regnans* and *E. rhodantha* [2, 7]. These studies have indicated that genetic diversity in the pollen pools of these species was produced by differences in flowering phenology. Observed low airborn pollen density in period 1 indicates that flowering intensity of trees that stands around sampled mother tree was low. In addition, mean wind velocity was twice as high during period 1 although the prevailing wind direction was similar during both periods. In period 1, both low levels of pollen supply from close

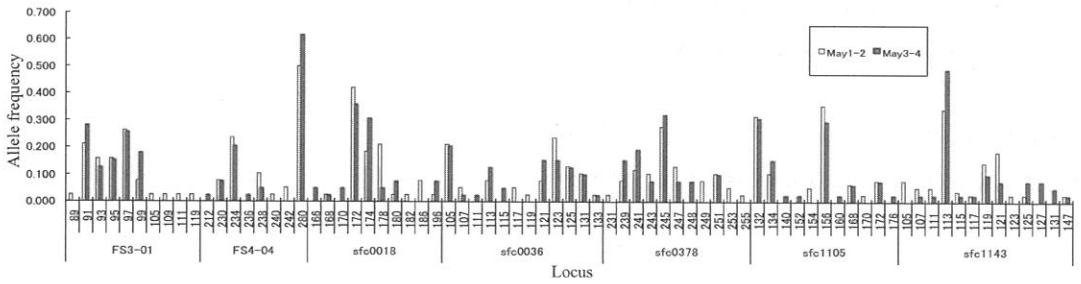

Fig.2 Detected frequencies of pollen alleles between 1-2 May (period 1) and 2-4 May (period 2).

range of sampled mother tree and strong wind that permit long-distance pollen dispersal might have contributed to increase genetic diversity of the pollen pool. However, further study is necessary in order to achieve quantitative evaluation of the factors that affect temporal variation in the pollen pool of *F. crenata.*

In conclusion, the results of this study indicate a high likelihood of temporal variation in genetic diversity of the pollen pool existing in populations of the species *F. crenata.* Furthermore, diversity in the pollen pool of wind-pollinated species may be caused, in part, by the temporal heterogeneity of pollen-mediated gene dispersal.

Acknowledgement

We thank J. Yuzurihara, Y. Hakamada, D. Ito and other members of the laboratory of Forest Ecology at Gifu University for their help conducting field studies.

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